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71 Applicant: **NITTO CHEMICAL INDUSTRY CO.,  
LTD.**  
**No. 5-1, Marunouchi 1-chome Chiyoda-ku  
Tokyo(JP)**

Applicant: **Beppu, Teruhiko**  
**No. 5-21, 1-chome**  
**Horinouchi Suginami-ku, Tokyo(JP)**

Applicant: **YAMADA, Hideaki**  
**19-1 Matsugasaki-Kinomoto-cho Sakyo-ku**  
**Kyoto-shi Kyoto-Fu(JP)**

72 Inventor: **Teruhiko, Beppu**  
**5-21, Horinouchi 1-chome, Suginami-ku,**  
**Tokyo(JP)**  
Inventor: **Hideaki, Yamada**  
**19-1, Matsugasaki Kinomotocho, Sakyo-ku,**  
**Kyoto-shi, Kyoto-fu(JP)**  
Inventor: **Toru, Nagasawa**  
**1-7, Takano Higashihirakicho, Sakyo-ku,**  
**Kyoto-shi, Kyoto-fu(JP)**  
Inventor: **Sueharu Horinouchi**  
**3-16-403 Etsuchujima 1-chome, Koutou-ku,**  
**Tokyo(JP)**  
Inventor: **Makoto, Nishiyama**  
**16-11, Nishiokuchi 2-chome, Shinjuku-ku,**  
**Tokyo(JP)**

74 Representative: **Vossius & Partner**  
**Siebertstrasse 4 P.O. Box 86 07 67**  
**W-8000 München 86(DE)**

54 DNA fragment encoding a polypeptide having nitrile hydratase activity, a transformant containing the DNA fragment and a process for the production of amides using the transformant.

57 The present invention discloses the amino acid sequence and nucleotide sequence of the  $\alpha$ - and  $\beta$ -subunits of two types of nitrile hydratase derived from Rhodococcus rhodochrous J-1. The DNA fragment encoding nitrile hydratase is inserted into an expression vector and the recombinant vector is used for transformation. The transformant contains multiple copies of the gene and can produce much higher levels of nitrile hydratase than conventionally used microorganisms.

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The present invention relates to a DNA fragment derived from Rhodococcus rhodochrous J-1 and encoding a polypeptide having nitrile hydratase activity which hydrates nitriles to amides. The invention also relates to a recombinant DNA containing the above DNA fragment, and a transformant transformed with the recombinant DNA. The present invention further relates to a method of producing nitrile hydratase using the transformant and of amides using nitrile hydratase.

Nitrile hydratase or nitrilase is known as an enzyme that hydrates nitriles to amides. Microorganisms that produce nitrile hydratase include those belonging to the genus Bacillus, the genus Bacteridium, the genus Micrococcus and the genus Brevibacterium (See, JP-B-62-21517/1989, USP No. 4,001,081), the genus Corynebacterium and the genus Nocardia (See, JP-B-56-17918/1981, USP No. 4,248,968), the genus Pseudomonas (See, JP-B-59-37951/1984, USP No. 4,637,982), the genus Rhodococcus, the genus Arthrobacter and the genus Microbacterium (See, JP-A-61-162193/1986, EP-A-0188316), and Rhodococcus rhodochrous (See, JP-A-2-470/1990, EP-A-0307926).

Nitrile hydratase has been used to hydrate nitriles to amides. In the invention, microorganisms are engineered to contain multiple copies of a recombinant DNA encoding nitrile hydratase according to a recombinant DNA technology. The recombinant produces a remarkably high level of nitrile hydratase compared with conventionally used microorganisms.

The present inventors previously disclosed a DNA fragment derived from Rhodococcus sp. N-774 (FERM BP-1936) which also encodes a polypeptide having nitrile hydratase activity (JP-A-2-119778/1988).

In contrast, the present inventors utilizes a DNA fragment derived from Rhodococcus rhodochrous J-1 for the production of nitrile hydratase. We isolated the gene encoding nitrile hydratase, inserted the gene into a suitable plasmid vector and transformed an appropriate host with the recombinant plasmid, thus successfully obtained the transformant producing nitrile hydratase which has high activity also on aromatic nitriles.

The present invention relates to

- (1) a DNA<sup>(H)</sup> fragment encoding a polypeptide having nitrile hydratase activity, said polypeptide comprising the  $\alpha^{(H)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 1 and the  $\beta^{(H)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 2;
- (2) a DNA<sup>(L)</sup> fragment encoding a polypeptide having nitrile hydratase activity, said polypeptide comprising the  $\alpha^{(L)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 3 and the  $\beta^{(L)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 4;
- (3) the DNA<sup>(H)</sup> fragment of (1) which contains a nucleotide sequence encoding said  $\alpha^{(H)}$ - and  $\beta^{(H)}$ -subunits, comprising the DNA sequence of the  $\alpha^{(H)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 5 and the DNA sequence of the  $\beta^{(H)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 6;
- (4) the DNA<sup>(L)</sup> fragment of (2) which contains a nucleotide sequence encoding said  $\alpha^{(L)}$ - and  $\beta^{(L)}$ -subunits, comprising the DNA sequence of the  $\alpha^{(L)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 7 and the DNA sequence of the  $\beta^{(L)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 8;
- (5) a recombinant DNA comprising the DNA<sup>(H)</sup> or the DNA<sup>(L)</sup> of (1)-(4) in a vector;
- (6) a transformant transformed with the recombinant DNA of (5);
- (7) a method for the production of nitrile hydratase which comprises culturing the transformant as described in (6) and recovering nitrile hydratase from the culture;
- (8) a method for the production of amides which comprises hydrating nitriles using nitrile hydratase as described in (7) to form amides; and
- (9) a method for the production of amides which comprises culturing the transformant as described in (6), and hydrating nitriles using the resultant culture, isolated bacterial cells, treated matter thereof, or a fixed material of them, to form amides.

The present invention is described in detail as follows.

The present invention is carried out by the steps (1)-(8):

#### (1) Isolation and Purification of Nitrile Hydratase and Partial Amino Acid Sequencing of Nitrile Hydratase

Two types of nitrile hydratase (designated as H type and L type, respectively) are isolated and purified from Rhodococcus rhodochrous J-1 (FERM BP-1478) and the both enzymes are separated into  $\alpha$  and  $\beta$  subunits using HPLC. N-Terminal amino acid sequence each of the subunits is determined and shown in the Sequence Listing by SEQ ID: Nos. 9-12.

#### (2) Preparation of a DNA Probe for a Nitrile Hydratase Gene

A DNA probe is prepared from JM105/pYUK121 (FERM BP-1937) as described in JP-A-2-119778/1990 due to the high degree of homology in the amino acid sequence between the nitrile hydratase  $\beta$  subunit of *Rhodococcus* sp. N-774 described in said Japanese Patent Official Gazette and those of *Rhodococcus rhodochrous* J-1. Plasmid pYUK121 containing nitrile hydratase gene derived from *Rhodococcus* sp. N-774  
 5 is prepared from a JM105/pYUK121 culture. pYUK121 DNA is digested with SphI and SalI. The SphI-SalI fragment contains the nitrile hydratase gene (shown in the Sequence Listing by SEQ ID: No. 13) of *Rhodococcus* sp. N-774. The DNA fragment is radiolabeled.

10 (3) Detection of a DNA Segment Containing a Nitrile Hydratase Gene from the Chromosome of *Rhodococcus rhodochrous* J-1

Chromosomal DNA is prepared from a *Rhodococcus rhodochrous* J-1 culture. The chromosomal DNA is digested with restriction enzymes and hybridized to the probe described in (2) using the Southern hybridization method [Southern, E.M., J. Mol. Biol. 98, 503 (1975)].

15 Two DNA fragments of a different length are screened.

(4) Construction of a Recombinant Plasmid

A recombinant plasmid is constructed by inserting the chromosomal DNA fragment as prepared in (3)  
 20 into a plasmid vector.

(5) Transformation and Screening for a Transformant Containing the Recombinant Plasmid

Transformants are prepared using the recombinant plasmid as described in (4). The transformant  
 25 containing the recombinant plasmid is selected using the probe as described in (2) according to the colony hybridization method [R. Bruce Wallace et. al., Nuc. Ac. Res. 9, 879 (1981)]. Additionally, the presence of the nitrile hydratase gene in the recombinant plasmid is confirmed using the Southern hybridization method. The plasmids thus selected are designated as pNHJ10H and pNHJ20L.

30 (6) Isolation and Purification of Plasmid DNA and Construction of the Restriction Map

Plasmid DNAs of pNHJ10H and pNHJ20L as prepared in (5) are isolated and purified. The restriction map of the DNAs is constructed (Fig. 1) to determine the region containing nitrile hydratase gene.

35 (7) DNA Sequencing

The extra segment of the inserted DNA fragment in pNHJ10H and pNHJ20L is excised using an appropriate restriction enzyme. The inserted DNA fragment is then used for sequencing. The nucleotide sequence of the DNA fragment (SEQ: ID Nos. 14, 15) reveals that it contains the sequence deduced from  
 40 the amino acid sequence as described in (1).

(8) Production of Nitrile Hydratase Using the Transformant and Conversion of Nitriles to Amides

The transformant as described in (8) is cultured. The bacterial cells are mixed with nitriles, a substrate  
 45 of nitrile hydratase, and amides are produced.

*Rhodococcus rhodochrous* J-1 was deposited with Fermentation Research Institute, Agency of Industrial Science and Technology, and was assigned the accession number FERM BP-1478. A transformant TGI/pNHJ10H containing pNHJ10H as described in (5) and a transformant TGI/pNHJ20L containing pNHJ20L as described in (5) were deposited with the above and assigned the accession number FERM BP-  
 50 2777 and FERM BP-2778, respectively.

Any vectors including a plasmid vector (e.g., pAT153, pMP9, pHC624, pKC7, etc.), a phage vector (e.g.,  $\lambda$ gt11 (Toyobo), Charon 4A (Amersham), etc.) may be used. Enzymes which may be used include SphI, SalI, EcoRI, BamHI, SacI, and the like, which are commercially available (Takara Shuzo). Various hosts may be used for transformation including but not limited to *E. coli* JM105 and *E. coli* TGI.

55 Culture media for the transformant are those ordinarily used in the art.

Conversion of nitriles to amides is carried out using nitrile hydratase, crude nitrile hydratase, the culture of the transformant, the isolated bacterial cells or treated matter thereof, and the like, prepared from the culture of the transformant.

Suitable nitriles in the invention include aromatic nitriles having 4-10 carbon atoms in the aromatic moiety and aliphatic nitriles having 2-6 carbon atoms, which are described in the European Patent Publication No. 0,307,926. Typical examples of the nitriles are 4-, 3- and 2-cyanopyridines, benzonitrile, 2,6-difluorobenzonitrile, 2-thiophene carbonitrile, 2-furonitrile, cyanopyrazine, acrylonitrile, methacrylonitrile, crotonitrile, acetonitrile and 3-hydroxypropionitrile.

The present invention discloses the amino acid sequence and nucleotide sequence of the  $\alpha$ - and  $\beta$ -subunits of two types of nitrile hydratase derived from *Rhodococcus rhodochrous* J-1. The DNA fragment encoding nitrile hydratase is inserted into an expression vector and the recombinant vector is used for transformation. The transformant contains multiple copies of the gene and can produce much higher levels of nitrile hydratase than conventionally used microorganisms.

Fig. 1 shows restriction maps of recombinant plasmids, pNHJ10H and pNHJ20L.

The present invention is illustrated by the following Example.

The following abbreviations are used in the Example.

TE:	Tris-HCl (10 mM; pH 7.8), EDTA (1 mM, pH 8.0)
TNE:	Tris-HCl (50 mM; pH 8.0), EDTA (1 mM, pH 8.0), NaCl (50 mM)
STE:	Tris-HCl (50 mM; pH 8.0), EDTA (5 mM, pH 8.0), Sucrose (35 mM)
2xYT medium:	1.6% Trypton; 1.0% Yeast extract, 0.5% NaCl

### Example

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#### (1) Isolation and Purification of Nitrile Hydratase and Partial Amino Acid Sequencing of Nitrile Hydratase

*Rhodococcus rhodochrous* J-1 was cultured in a medium (3 g/l of yeast extract, 0.5 g/l of  $\text{KH}_2\text{PO}_4$ , 0.5 g/l of  $\text{K}_2\text{HPO}_4$ , 0.5 g/l of  $\text{MgSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.01 g/l of  $\text{CoCl}_2$ , and 3 g/l of crotonamide, pH 7.2) at 28° C for 80 hours. The bacterial cells were harvested. 50 g of the bacterial cells was disrupted and fractionated with ammonium sulfate. The sample was dialyzed and the dialysate was centrifuged. The supernatant was loaded on DEAE-Cellulofine chromatography, Phenyl-Sepharose chromatography, Sephadex G-150 chromatography and Octyl-Sepharose chromatography. Two fractions with enzyme activity were obtained and dialyzed. The dialysates were loaded on a high performance liquid chromatography using a reversed phase column (Senshu Pak VP-304-1251, Senshu Kagaku), and two respective subunits ( $\alpha$  and  $\beta$ ) were obtained. N-terminal amino acid sequence of  $\alpha_1^{(H)}$ -,  $\beta_1^{(H)}$ -,  $\alpha_1^{(L)}$ - and  $\beta_1^{(L)}$ -subunits was determined using an Applied Biosystems model 470A protein sequencer. The amino acid sequences are shown in the Sequence Listing by SEQ ID: Nos. 9-12.

#### (2) Preparation of a DNA Probe for Nitrile Hydratase Gene

*E. coli* JM105 (FERM BP-1937) containing pYUK121 was cultured in 100 ml of 2xYT medium containing 50  $\mu\text{g/ml}$  of ampicillin at 30° C overnight (12 hours). The bacterial cells were harvested and TNE was added to the cells. The cell suspension was then centrifuged. 8 ml of STE and 10 mg of lysozyme were added to the pellet. The mixture was incubated at 0° C for five minutes followed by the addition of 4 ml of 0.25M EDTA. 2 ml of 10% SDS and 5 ml of 5M NaCl were then added to the mixture at room temperature. The resultant mixture was incubated at 0-4° C for three hours and then ultracentrifuged. 1/2 volume of 30% PEG 6000 was added to the supernatant. The mixture was incubated at 0-4° C overnight (12 hours) and centrifuged. TNE was added to the pellet to bring the volume to 7.5 ml and CsCl was then added to the suspension. The mixture was centrifuged to remove proteins. Then, 300-500 mg/ml of ethidium bromide was added to the supernatant. The mixture was transferred to a centrifuge tube. The tube was heat-sealed and then ultracentrifuged. cccDNA was extracted using a peristaltic pump. A bit more than equal amount of isopropyl alcohol saturated with water was added to the extract to rid of ethidium bromide. The sample was dialyzed against TE. About 3 ml of purified pYUK121 was obtained.

pYUK121 DNA was digested with SphI and Sall, resulting in a 2.07 kb DNA fragment containing a nitrile hydratase gene derived from *Rhodococcus* sp. N-774. The fragment was radiolabeled with  $^{32}\text{P}$  to produce a probe. The nucleotide sequence of the probe is shown in the Sequence Listing by SEQ ID: No. 13.

#### (3) Preparation of a DNA Fragment Containing a Nitrile Hydratase Gene of Chromosome

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*Rhodococcus rhodochrous* J-1 was cultured in 100 ml of a medium (10 g/l of glucose, 0.5 g/l of  $\text{KH}_2\text{PO}_4$ , 0.5 g/l of  $\text{K}_2\text{HPO}_4$ , 0.5 g/l of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1 g/l of yeast extract, 7.5 g/l of peptone, 0.01 g/l of  $\text{CoCl}_2$ , 7.5 g/l of urea, 1% glycine or 0.2  $\mu\text{g/ml}$  of ampicillin, 1 l of water, pH 7.2). The bacterial cells



were harvested and the pellet was washed with TNE. The pellet was then suspended in 10 ml of TE. 4 ml of 0.25M EDTA, 10-20 mg of lysozyme, 10-20 mg of achromoprotease and 10 ml of 10×SDS were added to the suspension. The suspension was incubated at 37° C for three hours. 15 ml of phenol was added to the suspension. The mixture was incubated at room temperature for 15 minutes and then centrifuged. The upper layer was removed, and 0.7 ml of 2.5M sodium acetate and diethyl ether were added to the supernatant. The mixture was centrifuged and the upper layer was discarded. Two volumes of ethanol were added to the bottom layer and DNA was removed with a glass rod. DNA was rinsed for five minutes each with TE:ethanol 2:8, 1:9, and 0:10 (v/v). DNA was then resuspended in 2-4 ml of TE (37° C). 10 µl of a mixture of RNase A and T<sub>1</sub> was added to the suspension and the mixture was incubated at 37° C. An equal amount of phenol was added to the mixture which was then centrifuged. More than equal amount of ether was added to the supernatant. The mixture was centrifuged again, and the upper layer was discarded and the bottom layer was saved. The bottom layer was dialyzed against 2 l of TE containing a small amount of chloroform overnight and further dialyzed against fresh TE for 3-4 hours. 4 ml of crude chromosomal DNA was obtained.

10 µl of TE, 3 µl of reaction buffer (10×) and 2 µl of SacI were added to 15 µl of crude chromosomal DNA. The mixture was incubated at 37° C for an hour and electrophoresed on an agarose gel at 60 V for three hours. The Southern hybridization of chromosomal DNA was carried out using the probe as described in (2). About 6.0 kb and 9.4 kb fragments were found to show a strong hybridization.

15 µl of chromosomal DNA was digested with SacI and electrophoresed on an agarose gel, as described above. 6.0 kb and 9.4 kb DNA fragments were cut out from the gel and taken in three volumes each of 8M NaClO<sub>4</sub>. After solubilization, each solution was dotted on GF/C (Whatman) filter paper (6 mm in diameter). Ten drops (≈ 100 µl) of TE containing 6M NaClO<sub>4</sub> and then ten drops (≈ 100 µl) of 95% ethanol were added to the filter paper. The paper was air-dried for 3 minutes and placed in 0.5 ml Eppendorf tube. 40 µl of TE was added to the tube and the whole was incubated at 47° C for 30 minutes. The tube was then centrifuged. About 40 µl of the supernatant was obtained which contained 6.0 kb and 9.4 kb DNA fragments containing a nitrile hydratase gene of chromosomal DNA.

The method of inserting the 6.0 kb DNA fragment into a vector is described below. The same method is applied for the insertion of the 9.4 kb DNA fragment into a vector.

#### 30 (4) Insertion of the Chromosomal DNA Fragment into a Vector

10 µl of TE, 3 µl of reaction buffer (10×) and 2 µl of SacI was added to 10 µl of pUC19. The mixture was incubated at 30° C for an hour. 2 µl of 0.25M EDTA was added to the mixture to stop the reaction. Then, 7 µl of 1M Tris-HCl (pH 9) and 3 µl of BAP (bacterial alkaline phosphatase) were added to the mixture. The mixture was incubated at 65° C for an hour. TE was then added to the mixture to make a total volume to 100 µl. The mixture was extracted 3× with an equal amount of phenol. An equal amount of ether was added to the extract. The bottom layer was removed and 10 µl of 3M sodium acetate and 250 µl of ethanol were added to the bottom layer. The mixture was incubated at -80° C for 30 minutes, centrifuged, dried, and resuspended in TE.

5 µl of pUC19 DNA thus obtained and 40 µl of the 6.0 kb DNA fragment as described in (3) were mixed. 6 µl of ligation buffer, 6 µl of ATP (6 mg/ml) and 3 µl of T4 DNA ligase were added to the mixture. The mixture was incubated at 4° C overnight (12 hours) to produce the recombinant plasmid containing the 6.0 kb DNA fragment encoding the desired enzyme in the SacI site of pUC19.

#### 45 (5) Transformation and Screening of Transformants

*E. coli* TGI (Amersham) was inoculated into 10 ml of 2×YT medium and incubated at 37° C for 12 hours. After incubation, the resultant culture was added to fresh 2×YT medium to a concentration of 1%, and the mixture was incubated at 37° C for two hours. The culture was centrifuged and the pellet was suspended in 5 ml of cold 50 mM CaCl<sub>2</sub>. The suspension was placed on ice for 40 minutes and then centrifuged. 0.25 ml of cold 50 mM CaCl<sub>2</sub> and 60 µl of the recombinant DNA as described in (4) were added to the pellet. The mixture was incubated at 0° C for 40 minutes, heat-shocked at 42° C for two minutes, placed on ice for five minutes, and added to 10 ml of 2×YT medium. The mixture was incubated at 37° C for 90 minutes with shaking, then centrifuged. The pellet was suspended in 1 ml of 2×YT medium, and two 10 µl aliquots of the suspension were plated on a 2×YT agar plate containing 50 µg/ml of ampicillin separately. The plate was incubated at 37° C. The colony grown on the plate was selected by the colony hybridization method: The colony was transferred to a nitrocellulose filter and digested. The DNA was fixed on the filter and hybridized to the probe as described in (2). The filter was autoradiographed and

a recombinant colony was selected. Additionally, the presence of a nitrile hydratase gene in the transformant was confirmed according to the Southern hybridization method.

#### 5 (6) Isolation and Purification of Recombinant Plasmid and Construction of the Restriction Map of the Inserted DNA Fragments

The transformant selected as described in (5) was grown in 100 ml of 2xYT medium containing 50  $\mu$ g/ml of ampicillin at 37° C overnight (12 hours). The bacterial cells were harvested and TNE was added to the cells. The cells were collected again by centrifugation, and 8 ml of STE and 10 mg of lysozyme were  
10 added to the cells. The mixture was incubated at 0° C for five minutes. 4 ml of 0.25M EDTA, 2 ml of 10% SDS (at room temperature) and 5 ml of 5M NaCl were added to the mixture. The mixture was incubated at 0-4° C for three hours, and ultracentrifuged. 1/2 volume of 30% PEG 6000 was added to the supernatant. The mixture was incubated at 0-4° C overnight (12 hours) and centrifuged again. TNE was added to the pellet to bring the volume up to 7.5 ml. CsCl was added to the suspension to rid of proteins. Then, 300-500  
15 mg/ml of ethidium bromide was added to the supernatant and the mixture was transferred to a centrifuge tube. The tube was heat-sealed and ultracentrifuged. cccDNA was removed using a peristaltic pump. A bit more than equal amount of isopropyl alcohol saturated with water was added to cccDNA to remove ethidium bromide. The DNA sample was dialyzed against TE, resulting in about 3 ml of purified recombinant DNA. The recombinant plasmid thus obtained containing a 6.7 kb DNA fragment was designated as pNHJ10H  
20 (The recombinant plasmid containing a 9.4 kb DNA fragment was designated as pNHJ20L).

These plasmid DNAs were digested with EcoRI, BamHI, PstI, SacI and Sall. The restriction maps were constructed and are shown in Fig. 1.

#### 25 (7) DNA Sequencing

The location of a nitrile hydratase gene in the DNA fragment of pNHJ10H was determined according to the restriction map constructed and to the Southern hybridization method. An extra segment in pNHJ10H was cleaved off with PstI and Sall: The 6.0 kb DNA fragment resulted in 1.97 kb. Similarly, an extra  
30 segment in pNHJ20L was cleaved off with EcoRI and SacI: The 9.4 kb DNA fragment resulted in 1.73 kb.

These DNA fragments were sequenced by the Sanger method [Sanger, F., Science 214: 1205-1210 (1981)] using M13 phage vector. The nucleotide sequence of the 1.97 kb DNA fragment (pNHJ10H) and the 1.73 kb DNA fragment (pNHJ20L) are shown in the Sequence Listing by SEQ ID: No. 14 and SEQ ID: No. 15, respectively.

The amino acid sequence deduced from the nucleotide sequence was found fully identical to the amino  
35 acid sequence as determined in (1). The sequence analysis also revealed that the DNA fragment contained the sequence coding for the  $\alpha$ - and  $\beta$ -subunits.

#### 40 (8) Production of Nitrile Hydratase Using the Transformant and Conversion of Nitriles to Amides Using Nitrile Hydratase

TG1 /pNHJ10H and TG1/pNHJ20L were inoculated into 10 ml of 2xYT medium containing 50  $\mu$ g/ml of ampicillin and incubated at 30° C overnight (12 hours). 1 ml of the resultant culture was added to 100 ml of 2xYT medium (50  $\mu$ g/ml of ampicillin, 0.1 g of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ /l). The mixture was incubated at 30° C for 4 hours. IPTG was added to the mixture to a final concentration of 1 mM. The mixture was incubated at 30° C  
45 for 10 hours. After harvesting the cells, the cells were suspended in 5 ml of 0.1 M phosphate buffer (pH 7.5). The suspensions were disrupted by sonification for 5 min and centrifuged at 12,000  $\times$ g for 30 min. The resulting supernatants were used for the enzyme assay. The enzyme assay was carried out in a reaction mixture (12 ml) containing 50 mM potassium phosphate buffer (pH 7.5), 6 mM benzonitrile and an appropriate amount of the enzyme. The reaction was carried out at 20° C for 30 min and stopped by the  
50 addition of 0.2 ml 1 M HCl. The amount of benzamide formed in the reaction mixture was determined by HPLC. As a control, the mixture obtained by the same procedure as described above but from E. coli TG1 was used. The levels of nitrile hydratase activity in cell-free extracts of E. coli containing pNHJ10H and pNHJ20L were  $1.75 \times 10^{-3}$  and  $6.99 \times 10^{-3}$  units/mg, respectively, when cultured in 2xYT medium in the presence of  $\text{CoCl}_2$  and IPTG. Benzamide was found in the reaction mixture of TG1/pNHJ10H and pNHJ20L,  
55 whereas no benzamide was found in the reaction mixture of TG1.

(1) INFORMATION FOR SEQ ID NO: 1

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 203 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

## (ii) MOLECULE TYPE: Peptide

## (vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

## (ix) FEATURES

(A) OTHER INFORMATION

 $\alpha$ (H)-subunit

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1

```

      5      10      15
MetSerGluHisValAsnLysTyrThrGluTyrGluAlaArgThr
      20      25      30
LysAlaIleGluThrLeuLeuTyrGluArgGlyLeuIleThrPro
      35      40      45
AlaAlaValAspArgValValSerTyrTyrGluAsnGluIleGly
      50      55      60
ProMetGlyGlyAlaLysValValAlaLysSerTrpValAspPro
      65      70      75
GluTyrArgLysTrpLeuGluGluAspAlaThrAlaAlaMetAla
      80      85      90
SerLeuGlyTyrAlaGlyGluGlnAlaHisGlnIleSerAlaVal
      95      100      105
PheAsnAspSerGlnThrHisHisValValValCysThrLeuCys
      110      115      120
SerCysTyrProTrpProValLeuGlyLeuProProAlaTrpTyr
      125      130      135
LysSerMetGluTyrArgSerArgValValAlaAspProArgGly

```

5 ValLeuLysArgAspPheGlyPheAspIleProAspGluValGlu  
 ValArgValTrpAspSerSerSerGluIleArgTyrIleValIle  
 ProGluArgProAlaGlyThrAspGlyTrpSerGluGluGluLeu  
 ThrLysLeuValSerArgAspSerMetIleGlyValSerAsnAla  
 10 LeuThrProGlnGluValIleVal

15

(2) INFORMATION FOR SEQ ID NO: 2

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 229 amino acids

(B) TYPE: Amino acid

25

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

30

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

35

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

40

$\beta^{(H)}$ -subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

45

MetAspGlyIleHisAspThrGlyGlyMetThrGlyTyrGlyPro  
 ValProTyrGlnLysAspGluProPhePheHisTyrGluTrpGlu  
 GlyArgThrLeuSerIleLeuThrTrpMetHisLeuLysGlyIle  
 SerTrpTrpAspLysSerArgPhePheArgGluSerMetGlyAsn  
 GluAsnTyrValAsnGluIleArgAsnSerTyrTyrThrHisTrp

55



5 LeuSerAlaAlaGluArgIleLeuValAlaAspLysIleIleThr  
80 85 90  
10 GluGluGluArgLysHisArgValGlnGluIleLeuGluGlyArg  
95 100 105  
15 TyrThrAspArgLysProSerArgLysPheAspProAlaGlnIle  
110 115 120  
20 GluLysAlaIleGluArgLeuHisGluProHisSerLeuAlaLeu  
125 130 135  
25 ProGlyAlaGluProSerPheSerLeuGlyAspLysIleLysVal  
140 145 150  
30 LysSerMetAsnProLeuGlyHisThrArgCysProLysTyrVal  
155 160 165  
35 ArgAsnLysIleGlyGluIleValAlaTyrHisGlyCysGlnIle  
170 175 180  
40 TyrProGluSerSerSerAlaGlyLeuGlyAspAspProArgPro  
185 190 195  
45 LeuTyrThrValAlaPheSerAlaGlnGluLeuTrpGlyAspAsp  
200 205 210  
50 GlyAsnGlyLysAspValValCysValAspLeuTrpGluProTyr  
215 220 225  
55 LeuIleSerAla  
230

(3) INFORMATION FOR SEQ ID NO: 3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 207 amino acids

(B) TYPE: Amino acid

**(C) STRANDEDNESS:**

(D) TOPOLOGY: Linear

## (ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: *Rhodococcus rhodochrous*

(B) STRAIN: J-1 (FERM BP-1478)

(ix) **FEATURES**

(A) OTHER INFORMATION

 $\alpha^{(L)}$ -subunit

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

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```

      5      10      15
MetThrAlaHisAsnProValGlnGlyThrLeuProArgSerAsn
      20      25      30
GluGluIleAlaAlaArgValLysAlaMetGluAlaIleLeuVal
      35      40      45
AspLysGlyLeuIleSerThrAspAlaIleAspHisMetSerSer
      50      55      60
ValTyrGluAsnGluValGlyProGlnLeuGlyAlaLysIleVal
      65      70      75
AlaArgAlaTrpValAspProGluPheLysGlnArgLeuLeuThr
      80      85      90
AspAlaThrSerAlaCysArgGluMetGlyValGlyGlyMetGln
      95      100      105
GlyGluGluMetValValLeuGluAsnThrGlyThrValHisAsn
      110      115      120
MetValValCysThrLeuCysSerCysTyrProTrpProValLeu
      125      130      135
GlyLeuProProAsnTrpTyrLysTyrProAlaTyrArgAlaArg
      140      145      150
AlaValArgAspProArgGlyValLeuAlaGluPheGlyTyrThr
      155      160      165
ProAspProAspValGluIleArgIleTrpAspSerSerAlaGlu
      170      175      180
LeuArgTyrTrpValLeuProGlnArgProAlaGlyThrGluAsn
      185      190      195
PheThrGluGluGlnLeuAlaAspLeuValThrArgAspSerLeu
      200      205
IleGlyValSerValProThrThrProSerLysAla

```

(4) INFORMATION FOR SEQ ID NO: 4

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 226 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

## (ii) MOLECULE TYPE: Peptide

## (vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

## (ix) FEATURES

(A) OTHER INFORMATION

 $\beta^{(L)}$ -subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4

```

      5           10           15
MetAspGlyIleHisAspLeuGlyGlyArgAlaGlyLeuGlyPro
      20           25           30
IleLysProGluSerAspGluProValPheHisSerAspTrpGlu
      35           40           45
ArgSerValLeuThrMetPheProAlaMetAlaLeuAlaGlyAla
      50           55           60
PheAsnLeuAspGlnPheArgGlyAlaMetGluGlnIleProPro
      65           70           75
HisAspTyrLeuThrSerGlnTyrTyrGluHisTrpMetHisAla
      80           85           90
MetIleHisHisGlyIleGluAlaGlyIlePheAspSerAspGlu
      95          100          105
LeuAspArgArgThrGlnTyrTyrMetAspHisProAspAspThr
     110          115          120
ThrProThrArgGlnAspProGlnLeuValGluThrIleSerGln
     125          130          135
LeuIleThrHisGlyAlaAspTyrArgArgProThrAspThrGlu
     140          145          150
AlaAlaPheAlaValGlyAspLysValIleValArgSerAspAla
     155          160          165
SerProAsnThrHisThrArgArgAlaGlyTyrValArgGlyArg
     170          175          180
ValGlyGluValValAlaThrHisGlyAlaTyrValPheProAsp
     185          190          195
ThrAsnAlaLeuGlyAlaGlyGluSerProGluHisLeuTyrThr
     200          205          210
ValArgPheSerAlaThrGluLeuTrpGlyGluProAlaAlaPro
     215          220          225
AsnValValAsnHisIleAspValPheGluProTyrLeuLeuPro
Ala

```

(5) INFORMATION FOR SEQ ID NO: 5

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 609 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

## (ii) MOLECULE TYPE: Genomic DNA

## (vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

## (ix) FEATURES

(A) OTHER INFORMATION

 $\alpha^{(H)}$ -subunit

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5

```

      15      30      45
GTGAGCGAGCACGTCAATAAGTACACGGAGTACGAGGCACGTACC
      60      75      90
AAGGCGATCGAAACCTTGCTGTACGAGCGAGGGCTCATCACGCCC
     105     120     135
GCCGCGGTGCGACCGAGTCGTTTTCGTACTACGAGAACGAGATCGGC
     150     165     180
CCGATGGGCGGTGCCAAGGTCGTGGCCAAGTCCTGGGTGGACCCT
     195     210     225
GAGTACCGCAAGTGGCTCGAAGAGGACGCGACGGCCGCGATGGCG
     240     255     270
TCATTGGGCTATGCCGGTGAGCAGGCACACCAAATTTCCGGCGGTC
     285     300     315
TTCAACGACTCCCAAACGCATCACGTGGTGGTGTGCACTCTGTGT
     330     345     360
TCGTGCTATCCGTGGCCGGTGCTTGGTCTCCCGCCCGCCTGGTAC
     375     390     405
AAGAGCATGGAGTACCGGTCCCGAGTGGTAGCGGACCCTCGTGGA
     420     435     450
GTGCTCAAGCGCGATTTCGGTTTTCGACATCCCGGATGAGGTGGAG

```



5 GTCAGGGTTTGGGACAGCAGCTCCGAAATCCGCTACATCGTCATC  
 CCGGAACGGCCGGCCGGCACCAGCGGTTGGTCCGAGGAGGAGCTG  
 10 ACCAAGCTGGTGAGCCGGGACTCGATGATCGGTGTCAGTAATGCG  
 CTCACACCGCAGGAAGTGATCGTA

(6) INFORMATION FOR SEQ ID NO: 6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 687 nucleic acids

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(H)}$ -subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6

45 ATGGATGGTATCCACGACACAGGCGGCATGACCGGATACGGACCG  
 GTCCCCTATCAGAAGGACGAGCCCTTCTTCCACTACGAGTGGGAG  
 GGTCCGACCCTGTCAATTCTGACTTGGATGCATCTCAAGGGCATA  
 50 TCGTGGTGGGACAAGTCGCGGTTCTTCCGGGAGTCGATGGGGAAC  
 GAAACTACGTCAACGAGATTGGCAACTCGTACTACACCCACTGG  
 55 CTGAGTGCGGCAGAACGTATCCTCGTCCCGACAAGATCATCACC

5 GAAGAAGAGCGAAAGCACCGTGTGCAAGAGATCCTTGAGGGTCCG  
 TACACGGACAGGAAGCCGTCGCGGAAGTTCGATCCGGCCCAGATC  
 10 GAGAAGGCGATCGAACGGCTTCACGAGCCCCACTCCCTAGCGCTT  
 CCAGGAGCGGAGCCGAGTTTCTCTCTCGGTGACAAGATCAAAGTG  
 AAGAGTATGAACCCGCTGGGACACACACGGTGCCCGAAATATGTG  
 15 CGGAACAAGATCGGGGAAATCGTCGCCTACCACGGCTGCCAGATC  
 TATCCCGAGAGCAGCTCCGCCGGCCTCGGCGACGATCCTCGCCCG  
 20 CTCTACACGGTCGCGTTTTTCCGCCCAGGAAGTGTGGGGCGACGAC  
 GGAAACGGGAAAGACGTAGTGTGCGTCGATCTCTGGGAACCGTAC  
 25 CTGATCTCTGCG

(7) INFORMATION FOR SEQ ID NO: 7

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 621 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: *Rhodococcus rhodochrous* J-1

(FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\alpha^{(L)}$ -subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7

5 ATGACCGCCCACAATCCCGTCCAGGGCACGTTGCCACGATCGAAC  
GAGGAGATCGCCGCACGCGTGAAGGCCATGGAGGCCATCCTCGTC  
10 GACAAGGGCCTGATCTCCACCGACGCCATCGACCACATGTCCTCG  
GTCTACGAGAACGAGGTGGTCTCAACTCGGCGCCAAGATCGTC  
GCCCCGCGCTGGGTGCGATCCCGAGTTCAAGCAGCGCCTGCTCACC  
15 GACGCCACCAGCGCCTGCCGTGAAATGGGCGTCGGCGGCATGCAG  
GGCGAAGAAATGGTCTGTGCTGGAAAACACCGGCACGGTCCACAAC  
20 ATGGTCTGTATGTACCTTGTGCTCGTGTATCCGTGGCCGGTTCTC  
GGCCTGCCACCCAACTGGTACAAGTACCCCGCCTACCGCGCCCGC  
GCTGTCCGCGACCCCGAGGTGTGCTGGCCGAATTCCGGATATACC  
25 CCGGACCCTGACGTGAGATCCGGATATGGGACTCGAGTGCCGAA  
CTTCGCTACTGGGTCTGCGCAACGCCCGGCGGACCGAGAAC  
30 TTCACCGAAGAACAACCTCGCCGACCTCGTCAACCGCGACTCGCTC  
ATCGGCGTATCCGTCCCCACCACACCCAGCAAGGCC

(8) INFORMATION FOR SEQ ID NO: 8

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 678 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: *Rhodococcus rhodochrous*

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(L)}$ -subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8

```

15      15      30      45
ATGGATGGAATCCACGACCTCGGTGGCCGCCGCCGCTGGGTCCG
      60      75      90
ATCAAGCCCGAATCCGATGAACCTGTTTTCCATTCCGATTGGGAG
      105     120     135
CGGTCCGTTTTTGACGATGTTCCCGGCGATGGCGCTGGCCGGCGCG
20      150     165     180
TTCAATCTCGACCAGTTCCGGGGCGCGATGGAGCAGATCCCCCGG
      195     210     225
CACGACTACCTGACCTCGCAATACTACGAGCACTGGATGCACGCG
25      240     255     270
ATGATCCACCACGGCATCGAGGCGGGCATCTTCGATTCCGACGAA
      285     300     315
CTCGACCGCCGCACCCAGTACTACATGGACCATCCGGACGACACG
30      330     345     360
ACCCCCACGCGGCAGGATCCGCAACTGGTGGAGACGATCTCGCAA
      375     390     405
CTGATCACCACGGAGCCGATTACCGACGCCCGACCGACACCGAG
      420     435     450
GCCGCATTCCGCGTAGGCGACAAAGTCATCGTGCGGTCCGACGCC
35      465     480     495
TCACCGAACACCCACACCCGCCGCCGGATACGTCCGCGGTCTGT
      510     525     540
GTCGGCGAAGTCGTGGCGACCCACGGCGCGGTATGTCTTTCCGGAC
40      555     570     585
ACCAACGCACTCGGCGCCGGCGAAGCCCCGAACACCTGTACACC
      600     615     630
GTGCGGTTCTCGGCGACCGAGTTGTGGGGTGAACCTGCCGCCCG
45      645     660     675
AACGTCGTCAATCACATCGACGTGTTCGAACCGTATCTGCTACCG
GCC

```

(9) INFORMATION FOR SEQ ID NO: 9



(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 26 amino acids

(B) TYPE: Amino acid

10 (C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

15 (vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

20 (B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

25  $\alpha^{(H)}$ -subunit:  $\alpha_1^{(H)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9

30 Ser-Glu-His-Val-Asn<sup>5</sup>-Lys-Tyr-Thr-Glu<sup>10</sup>-Tyr-Glu-Ala-Arg-Thr-Lys<sup>15</sup>  
Ala-Ile-Glu-Thr<sup>20</sup>-Leu-Leu-Tyr-Glu<sup>25</sup>-Arg-Gly-Leu

35 (10) INFORMATION FOR SEQ ID NO: 10

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 28 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

45 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

50 (vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

55

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(H)}$ -subunit:  $\beta_1^{(H)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10

Met-Asp-Gly-Ile-His-Asp-Thr-Gly-Gly-Met-Thr-Gly-Tyr-Gly-Pro  
Val-Pro-Tyr-Gln-Lys-Asp-Glu-Pro-Phe-Phe-His-Tyr-Glu

(11) INFORMATION FOR SEQ ID NO: 11

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\alpha^{(L)}$ -subunit:  $\alpha_1^{(L)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11

Thr-Ala-His-Asn-Pro-Val-Gln-Gly-Thr-Leu-Pro-Arg-?-Asn-Glu

5

(12) INFORMATION FOR SEQ ID NO: 12

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids

15

(B) TYPE: Amino acid

(C) STRANDEDNESS:

20

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

25

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

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(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(L)}$ -subunit:  $\beta_1^{(L)}$

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12

40

Met-Asp-Gly-Ile-His-Asp-Leu-Gly-Gly-Arg-Ala-?-Leu-?-Pro  
Ile-Lys-Pro-Glu

45

(13) INFORMATION FOR SEQ ID NO: 13

50

(i) SEQUENCE CHARACTERISTICS:

55

(A) LENGTH: 2070 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus sp.

(B) STRAIN: N-774 (FERM BP-1936)

(ix) FEATURES

from nucleotide No. 675 to 1295: subunit  $\alpha$

from nucleotide No. 1225 to 1960: subunit  $\beta$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13

SphI

GCATGCTTTCCACATCTGGAACGTGATCGCCACGGACGGTGGTG  
 CCTACCAGATGTTGGACGGCAACGGATACGGCATGAACGCCGAAG  
 GTTTGTACGATCCGGAACTGATGGCACACTTTGCTTCTCGACGCA  
 TTCAGCACGCCGACGCTCTGTCCGAAACCGTCAAACCTGGTGGCCC  
 TGACCGGCCACACGGCATCACCAACCTCGGCGGCGCGAGCTACG  
 GCAAAGCCCGGAACCTCGTACCGCTTGCCCGCGCCGCCTACGACA  
 CTGCCTTGAGACAATTGACGTCCTGGTGATGCCAACGCTGCCCT  
 ACGTCGCATCCGAATTGCCGGCGGAAGGACGTAGATCGTGCAACCT



TCATCACCAAGGCTCTCGGGATGATCGCCAACACGGGCACCA<sup>400</sup>TTCG  
 5 ACGTGACCGGACATCCGTCCCTGTCCGTTCCGGCCGGCCTGGTGA  
 .<sup>450</sup>  
 ACGGGGTTCCGGTCGGAATGATGATCACCGGCAGACACTTCGACG  
 .<sup>500</sup> Hind III  
 10 ATGCGACAGTCCTTCGTGTCCGACGCGCATTTCGAAAAGCTTCGCG  
 .<sup>550</sup>  
 GCCGGTTTCCGACGCGCGCGCGAACGCGCCTCCA<sup>600</sup>ACTCTGCACCAC  
 AACTCAGCCCCCGCCTAGTCCTGACGCACTGTCAGACAACAAATTC  
 15 CACCGATTCAACACATGATCAGCCACATAAGAAAAGGTGAACCAG  
 .<sup>650</sup>  
 ATGTCAGTAACGATCGACCACACAACGGAGAACGCCCGCACCGGCC  
 MetSerValThrIleAspHisThrThrGluAsnAlaAlaProAla  
 20 . Subunit  $\alpha$   
 CAGGCGGCGGTCTCCGACCGGGCGGTGGGCACTGTTCCGCGCACTC  
 GlnAlaAlaValSerAspArgAlaTrpAlaLeuPheArgAlaLeu  
 .<sup>700</sup>  
 GACGGTAAGGGATTGGTACCCGACGGTTACGTTCGAGGGATGGAAG  
 25 AspGlyLysGlyLeuValProAspGlyTyrValGluGlyTrpLys  
 .<sup>750</sup>  
 AAGACCTCCGAGGAGGACTTCAGTCCAAGGCGCGGAGCGGAATTG  
 LysThrSerGluGluAspPheSerProArgArgGlyAlaGluLeu  
 30 . Pvu II  
 GTAGCGCGCGCATGGACCGACCCCGAGTTCCGGCAGCTGCTTCTC  
 ValAlaArgAlaTrpThrAspProGluPheArgGlnLeuLeuLeu  
 .<sup>800</sup> Kpn I  
 35 ACCGACGGTACCGCCGCGAGTTGCCCAGTACGGATACCTGGGCCCC  
 ThrAspGlyThrAlaAlaValAlaGlnTyrGlyTyrLeuGlyPro  
 .<sup>850</sup>  
 CAGGCGGCCTACATCGTGGCAGTCGAAGACACCCCGACACTCAAG  
 GlnAlaAlaTyrIleValAlaValGluAspThrProThrLeuLys  
 40 .<sup>900</sup>  
 AACGTGATCGTGTGCTCGCTGTGTTTCATGCACCGCGTGGCCCATC  
 AsnValIleValCysSerLeuCysSerCysThrAlaTrpProIle  
 .<sup>950</sup>  
 CTCGGTCTGCCACCCACCTGGTACAAGAGCTTCGAATACCGTGCG  
 45 LeuGlyLeuProProThrTrpTyrLysSerPheGluTyrArgAla

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CGCGTGGTCCGCGAACCACGGAAGGTTCTCTCCGAGATGGGAACC  
 ArgValValArgGluProArgLysValLeuSerGluMetGlyThr  
 5 GAGATCGCGTCCGACATCGAGATTGCGGTCTACGACACCACCGCC  
 GluIleAlaSerAspIleGluIleArgValTyrAspThrThrAla  
 GAAACTCGCTACATGGTCCTCCCGCAGCGTCCCGCCGGCACCGAA  
 10 GluThrArgTyrMetValLeuProGlnArgProAlaGlyThrGlu  
 Pst I  
 GGCTGGAGCCAGGAACAACCTGCAGGAAATCGTCACCAAGGACTGC  
 GlyTrpSerGlnGluGlnLeuGlnGluIleValThrLysAspCys  
 15 CTGATCGGGGTTGCAATCCCGCAGGTTCCCAACCGTCTGATCACCC  
 LeuIleGlyValAlaIleProGlnValProThrValTRM  
 CGACAAGAAGGAAGCACACC-ATGGATGGAGTACACGATCTTGCC  
 20 MetAspGlyValHisAspLeuAla  
 Subunit  $\beta$   
 GGAGTACAAGGCTTCGGCAAAGTCCCGCATACCGTCAACGCCGAC  
 GlyValGlnGlyPheGlyLysValProHisThrValAsnAlaAsp  
 25 ATCGGCCCCACCTTTCACGCCGAATGGGAACACCTGCCCTACAGC  
 IleGlyProThrPheHisAlaGluTrpGluHisLeuProTyrSer  
 CTGATGTTCCGCCGGTGTCCCGAAGTCCGGGGCTTCAGCGTCGAC  
 30 LeuMetPheAlaGlyValAlaGluLeuGlyAlaPheSerValAsp  
 GAAGTGCGATACGTCTCGTCCGAGCGGATGGAGCCGGGCCACTACATG  
 GluValArgTyrValValGluArgMetGluProGlyHisTyrMet  
 35 ATGACCCCGTACTACGAGAGGTACGTCATCGGTGTCCGACATTG  
 MetThrProTyrTyrGluArgTyrValIleGlyValAlaThrLeu  
 ATGGTCGAAAAGGGAATCCTGACGCAGGACGAACTCGAAAGCCTT  
 40 MetValGluLysGlyIleLeuThrGlnAspGluLeuGluSerLeu  
 GCGGGGGGACCGTTCCCACTGTCACGGCCCAAGCGAATCCGAAGGG  
 45 AlaGlyGlyProPheProLeuSerArgProSerGluSerGluGly  
 50  
 55

5 CGGCCGGCACCCTCGAGACGACCACCTTCGAAGTCGGGCAGCGA  
 ArgProAlaProValGluThrThrThrPheGluValGlyGlnArg  
 10 GTACCGGTACGCGACGAGTACGTTCCGGGGCATATTTCGAATGCCT  
 ValArgValArgAspGluTyrValProGlyHisIleArgMetPro  
 GCATACTGCCGTGGACGAGTGGGAACCATCTCTCATCGAACTACC  
 AlaTyrCysArgGlyArgValGlyThrIleSerHisArgThrThr  
 15 GAGAAGTGGCCGTTTCCCGACGCAATCGGCCACGGGCGCAACGAC  
 GluLysTrpProPheProAspAlaIleGlyHisGlyArgAsnAsp  
 GCCGGCGAAGAACCGACGTACCACGTGAAGTTCGCCGCCGAGGAA  
 AlaGlyGluGluProThrTyrHisValLysPheAlaAlaGluGlu  
 20 TTGTTCCGGTAGCGACACCGACGGTGGGAAGCGTCGTTGTCGACCTC  
 LeuPheGlySerAspThrAspGlyGlySerValValValAspLeu  
 TTCGAGGGTTACCTCGAGCCTGCGGCCTGATCTTCCAGCATTCCA  
 PheGluGlyTyrLeuGluProAlaAlaTRM  
 25 GGCGGCGGTCAACCGATCACAGCGGTTCTGTGCGACCGCCGCCTGA  
 TCACCACGATTCACTCATTGGAAGGACACTGGAAATCATGGTCCG  
 Sal I  
 AC

(14) INFORMATION FOR SEQ ID NO: 14

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1970 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous J-1

(FERM BP-1478)

## 5 (ix) FEATURES

from nucleotide No. 408 to 1094: subunit  $\beta^{(H)}$ 10 from nucleotide No. 1111 to 1719: subunit  $\alpha^{(H)}$ 

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14

15 CTGCAGCTCGAACATCGAAGGGTGGGAGCCGAGAGATCGGAGACGCAGACACCCGGAGGG  
 20 AACTTAGCCTCCCGGACCGATGCGTGTCTGGCAACGCCTCAAATTCAGTGCAAGCGAT  
 TCAATCTTGTTACTTCCAGAACCGAATCACGTCCCCGTAGTGTGCGGGGAGAGCGCCCGA  
 25 ACGCAGGGATGGTATCCATGCGCCCCTTCTCTTTTTCGAACGAGAACCGGCCGGTACAGCC  
 GACCCGGAGACACTGTGACCGCGTTCAACGATTGTTGTGCTGTGAAGGATTCACCCAAGC  
 30 CAACTGATATCGCCATTCCGTTGCCGGAACATTTGACACCTTCTCCCTACGAGTAGAAGC  
 CAGCTGGACCCCTCTTTGAGCCCAGCTCCGATGAAAGGAATGAGGAAATGGATGGTATCC  
 35 MetAspGlyIleH  
 Subunit  $\beta^{(H)}$   
 40 ACGACACAGGCGGCATGACCGGATACGGACCGGTCCCCTATCAGAAGGACGAGCCCTTCT  
 isAspThrGlyGlyMetThrGlyTyrGlyProValProTyrGlnLysAspGluProPheP  
 TCCACTACGAGTGGGAGGGTCCGACCCGTCAATTCTGACTTGGATGCATCTCAAGGGCA  
 heHisTyrGluTrpGluGlyArgThrLeuSerIleLeuThrTrpMetHisLeuLysGlyI  
 45 TATCGTGGTGGGACAAGTCGCGGTTCTTCCGGGAGTCCGATGGGGAACGAAACTACGTCA  
 leSerTrpTrpAspLysSerArgPhePheArgGluSerMetGlyAsnGluAsnTyrValA  
 ACGAGATTCGCAACTCGTACTACACCCACTGGCTGAGTGGGCAGAACGTATCCTCGTCC  
 snGluIleArgAsnSerTyrTyrThrHisTrpLeuSerAlaAlaGluArgIleLeuValA  
 50 CCGACAAGATCATCACCGAAGAAGAGCGAAAGCACCGTGTGCAAGAGATCCTTGAGGGTC  
 laAspLysIleIleThrGluGluGluArgLysHisArgValGlnGluIleLeuGluGlyA

55



730 740 750 760 770 780  
 GGTACACGGACAGGAAGCCGTCGCGGAAGTTCGATCCGGCCCAGATCGAGAAGGCGATCG  
 rgTyrThrAspArgLysProSerArgLysPheAspProAlaGlnIleGluLysAlaIleG  
 5 AACGGCTTCACGAGCCCCACTCCCTAGCGCTTCCAGGAGCGGAGCCGAGTTTCTCTCTCG  
 luArgLeuHisGluProHisSerLeuAlaLeuProGlyAlaGluProSerPheSerLeuG  
 850 860 870 880 890 900  
 GTGACAAGATCAAAGTGAAGAGTATGAACCCGCTGGGACACACACGGTGCCCGAAATATG  
 lyAspLysIleLysValLysSerMetAsnProLeuGlyHisThrArgCysProLysTyrV  
 10 TCGCGAACAAGATCGGGGAAATCGTCGCCTACCACGGCTGCCAGATCTATCCCGAGAGCA  
 alArgAsnLysIleGlyGluIleValAlaTyrHisGlyCysGlnIleTyrProGluSerS  
 910 920 930 940 950 960  
 GCTCCGCGCGCCTCGGGGACGATCCTCGCCCGCTCTACACGGTCGCGTTTTTCCGCCCAGG  
 15 erSerAlaGlyLeuGlyAspAspProArgProLeuTyrThrValAlaPheSerAlaGlnG  
 970 980 990 1000 1010 1020  
 AACTGTGGGGCGACGACGGAAACGGGAAAGACGTAGTGTGCGTCGATCTCTGGGAACCGT  
 luLeuTrpGlyAspAspGlyAsnGlyLysAspValValCysValAspLeuTrpGluProT  
 1030 1040 1050 1060 1070 1080  
 ACCTGATCTCTGCGTGAAAGGAATACGATAGTGAGCGAGCACGTCAATAAGTACACGGAG  
 20 yrLeuIleSerAla MetSerGluHisValAsnLysTyrThrGlu  
 Subunit  $\alpha$  (H)  
 1150 1160 1170 1180 1190 1200  
 TACGAGGCACGTACCAAGGCGATCGAAACCTTGCTGTACGAGCGAGGGCTCATCAGCCCC  
 25 TyrGluAlaArgThrLysAlaIleGluThrLeuLeuTyrGluArgGlyLeuIleThrPro  
 1210 1220 11230 1240 1250 1260  
 GCCGCGGTTCGACCGAGTCGTTTCGTACTACGAGAACGAGATCGGCCCGATGGGCGGTGCC  
 AlaAlaValAspArgValValSerTyrTyrGluAsnGluIleGlyProMetGlyGlyAla  
 1270 1280 1290 1300 1310 1320  
 AAGGTCGTGGCCAAGTCCTGGGTGGACCCTGAGTACCGCAAGTGGCTCGAAGAGGACGCG  
 30 LysValValAlaLysSerTrpValAspProGluTyrArgLysTrpLeuGluGluAspAla  
 1330 1340 1350 1360 1370 1380  
 ACGGCCGCGATGGCGTCATTGGGCTATGCCGGTGAGCAGGCACACCAAATTTCCGGCGGTC  
 35 ThrAlaAlaMetAlaSerLeuGlyTyrAlaGlyGluGlnAlaHisGlnIleSerAlaVal  
 1390 1400 1410 1420 1430 1440  
 TTCAACGACTCCCAAACGCATCACGTGGTGGTGTGCACTCTGTGTTCGTGCTATCCGTGG  
 PheAsnAspSerGlnThrHisHisValValValCysThrLeuCysSerCysTyrProTrp

1450 1460 1470 1480 1490 1500  
 CCGGTGCTTGGTCTCCCGCCCGCCTGGTACAAGAGCATGGAGTACCGGTCCCGAGTGCTA  
 ProValLeuGlyLeuProProAlaTrpTyrLysSerMetGluTyrArgSerArgValVal  
 5  
 1510 1520 1530 1540 1550 1560  
 GCGGACCCTCGTGGAGTGCTCAAGCGCGATTTCGGTTTCGACATCCCGGATGAGGTGGAG  
 AlaAspProArgGlyValLeuLysArgAspPheGlyPheAspIleProAspGluValGlu  
 10  
 1570 1580 1590 1600 1610 1620  
 GTCAGGGTTTGGGACAGCAGCTCCGAAATCCGCTACATCGTCATCCCGGAACGGCCGCC  
 ValArgValTrpAspSerSerSerGluIleArgTyrIleValIleProGluArgProAla  
 1630 1640 1650 1660 1670 1680  
 GGCACCGACGGTTGGTCCGAGGAGGAGCTGACGAAGCTGGTGAGCCGGGACTCGATGATC  
 GlyThrAspGlyTrpSerGluGluGluLeuThrLysLeuValSerArgAspSerMetIle  
 15  
 1690 1700 1710 1720 1730 1740  
 GGTGTCAGTAATGCGCTCACACCGCAGGAAGTGATCGTATGAGTGAAGACACACTCACTG  
 GlyValSerAsnAlaLeuThrProGlnGluValIleVal  
 1750 1760 1770 1780 1790 1800  
 ATCGGCTCCCGGCGACTGGGACCGCCGCACCGCCCCGCGACAATGGCGAGCTTGTATTCA  
 20  
 1810 1820 1830 1840 1850 1860  
 CCGAGCCTTGGGAAGCAACGGCATTCTGGGGTCGCCATCGCGCTTTCGGATCAGAAGTCGT  
 1870 1880 1890 1900 1910 1920  
 ACGAATGGGAGTTCTTCCGACAGCGTCTCATTCACTCCATCGCTGAGGCCAACGGTTGCG  
 25  
 1930 1940 1950 1960 1970  
 AGGCATACTACGAGAGCTGGACAAAGGCGCTCGAGGCCAGCGTGGTTCGAC

(15) INFORMATION FOR SEQ ID NO: 15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1731 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

from nucleotide No. 171 to 848: subunit  $\beta^{(L)}$

from nucleotide No. 915 to 1535: subunit  $\alpha^{(L)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15

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10      20      30      40      50      60
GAGCTCCCTGGAGCCACTCGCGCCGACGCATCCACGCTCGGACAGCCCACGGTGCGGATC

70      80      90      100     110     120
ACCCCTGTTTCGTCCGTAACAGAACAGTAACATGTCATCAGGTCATGACGTGTTGACGCAT

130     140     150     160     170     180
TAGACGAGGGCACATAGGGTTGGTGACTCACGGCACAAGGAGAGCATTTCATGGATGGAA
                                     MetAspGlyI
                                     Subunit  $\beta^{(L)}$ 

190     200     210     220     230     240
TCCACGACCTCGGTGGCCGCGCCGGCCTGGGTCCGATCAAGCCCGAATCCGATGAACCTG
leHisAspLeuGlyGlyArgAlaGlyLeuGlyProIleLysProGluSerAspGluProV

250     260     270     280     290     300
TTTTCCATTCCGATTGGGAGCGGTTCGTTTTTGACGATGTTCCCGGCGATGGCGCTGGCCG
aIPheHisSerAspTrpGluArgSerValLeuThrMetPheProAlaMetAlaLeuAlaG

310     320     330     340     350     360
GCGCGTTCAATCTCGACCAGTTCCGGGGCGCGATGGAGCAGATCCCCCGCACGACTACC
lyAlaPheAsnLeuAspGlnPheArgGlyAlaMetGluGlnIleProProHisAspTyrL

370     380     390     400     410     420
TGACCTCGCAATACTACGAGCACTGGATGCACGCGATGATCCACCACGGCATCGAGGCGG
euThrSerGlnTyrTyrGluHisTrpMetHisAlaMetIleHisHisGlyIleGluAlaG

430     440     450     460     470     480
GCATCTTCGATTCCGACGAACTCGACCGCCGCACCCAGTACTACATGGACCATCCGGACG
lyIlePheAspSerAspGluLeuAspArgArgThrGlnTyrTyrMetAspHisProAspA

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490 500 510 520 530 540  
 ACACGACCCCCACGCGGCAGGATCCGCAACTGGTGGAGACGATCTCGCAACTGATCACCC  
 spThrThrProThrArgGlnAspProGlnLeuValGluThrIleSerGlnLeuIleThrH  
 550 560 570 580 590 600  
 ACGGAGCCGATTACCGACGCCCGACCGACACCGAGGCCGATTCCGCCGTAGGCGACAAAG  
 isGlyAlaAspTyrArgArgProThrAspThrGluAlaAlaPheAlaValGlyAspLysV  
 610 620 630 640 650 660  
 TCATCGTGCGGTCCGACGCCTCACCGAACACCCACACCCGCCGCCGGATACGTCCGCG  
 10 alleleValArgSerAspAlaSerProAsnThrHisThrArgArgAlaGlyTyrValArgG  
 670 680 690 700 710 720  
 GTCGTGTCCGCGAAGTCGTGGCGACCCACGGCGCGTATGTCTTCCGGACACCAACGCAC  
 lyArgValGlyGluValValAlaThrHisGlyAlaTyrValPheProAspThrAsnAlaL  
 730 740 750 760 770 780  
 TCGGCGCCGCGGAAAGCCCCGAACACCTGTACACCGTGCGGTTCTCGGCGACCGAGTTGT  
 15 euGlyAlaGlyGluSerProGluHisLeuTyrThrValArgPheSerAlaThrGluLeuT  
 790 800 810 820 830 840  
 GGGGTGAACCTGCCGCCCGAACGTCGTCAATCACATCGACGTGTTCCAACCGTATCTGC  
 20 rpGlyGluProAlaAlaProAsnValValAsnHisIleAspValPheGluProTyrLeuL  
 850 860 870 880 890 900  
 TACCGGCCTGACCAGGTCATCCGGTCCACCCAGCGAGACGTCCCTTCACCACAGACAGAA  
 euProAla  
 910 920 930 940 950 960  
 ACGAGCCCACCCCGATGACCGCCCAATCCCGTCCAGGGCACGTTGCCACGATCGAACG  
 25 MetThrAlaHisAsnProValGlnGlyThrLeuProArgSerAsnG  
 Subunit  $\alpha$  (L)  
 970 980 990 1000 1010 1020  
 AGGAGATCGCCGCACGCGTGAAGGCCATGGAGGCCATCCTCGTCGACAAGGGCCTGATCT  
 30 luGluIleAlaAlaArgValLysAlaMetGluAlaIleLeuValAspLysGlyLeuIleS  
 1030 1040 1050 1060 1070 1080  
 CCACCGACGCCATCGACCACATGTCCTCGGTCTACGAGAACGAGGTCCGTCTCAACTCG  
 erThrAspAlaIleAspHisMetSerSerValTyrGluAsnGluValGlyProGlnLeuG  
 1090 1100 1110 1120 1130 1140  
 GCGCCAAGATCGTCGCCCGCGCCTGGGTTCGATCCCGAGTTCAAGCAGCGCCTGCTCACCG  
 35 lyAlaLysIleValAlaArgAlaTrpValAspProGluPheLysGlnArgLeuLeuThrA  
 1150 1160 1170 1180 1190 1200  
 ACGCCACCAGCGCCTGCCGTGAAATGGGCGTCCGGCGGCATGCAGGGCGAAGAAATGGTGC  
 spAlaThrSerAlaCysArgGluMetGlyValGlyGlyMetGlnGlyGluGluMetValV  
 1210 1220 1230 1240 1250 1260  
 TGCTGGAAAACACCGGCACGGTCCACAACATGGTTCGTATGTACCTTGTGCTCGTGCTATC  
 40 alLeuGluAsnThrGlyThrValHisAsnMetValValCysThrLeuCysSerCysTyrP  
 1270 1280 1290 1300 1310 1320  
 CGTGGCCGGTTCTCGGCCTGCCACCCAACTGGTACAAGTACCCCGCCTACCGCGCCCGCG  
 45 roTrpProValLeuGlyLeuProProAsnTrpTyrLysTyrProAlaTyrArgAlaArgA  
 1330 1340 1350 1360 1370 1380  
 CTGTCCGCGACCCCGAGGTGTGCTGGCCGAATTCCGATATACCCCGACCCTGACGTCCG  
 laValArgAspProArgGlyValLeuAlaGluPheGlyTyrThrProAspProAspValG

1390 1400 1410 1420 1430 1440  
 AGATCCGGATATGGGACTCGAGTGCCGAACCTTCGCTACTGGGTCTGCGCAACGCCACG  
 IuIleArgIleTrpAspSerSerAlaGluLeuArgTyrTrpValLeuProGlnArgProA  
 5 CCGGCACCGAGAACTTCACCGAAGAACAACCTCGCCGACCTCGTCACCCGCGACTCGCTCA  
 IaGlyThrGluAsnPheThrGluGluGlnLeuAlaAspLeuValThrArgAspSerLeuI  
 10 TCGGCGTATCCGTCCCCACCACACCCAGCAAGGCCTGACATGCCCCGACTCAACGAACAA  
 IeGlyValSerValProThrThrProSerLysAla  
 1500 1510 1520 1530 1540 1550 1560  
 CCCCACCCGGGTCTCGAAGCCAACCTCGGCGACCTGGTACAGAATCTGCCGTTCAACGAA  
 1600 1610 1620  
 CGAATCCCCCGCCGCTCCGGCGAGGTGCGCTTCGATCAGGCCTGGGAGATCCGCCCTTC  
 1630 1640 1650 1660 1670 1680  
 AGCATTGCCACCGCATTGCATGGCCAGGGCCGATTGCAATGGGACGAATTC  
 1690 1700 1710 1720 1730

# Claims

1. A DNA<sup>(H)</sup> fragment encoding a polypeptide having nitrile hydratase activity, said polypeptide comprising the  $\alpha^{(H)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 1 and the  $\beta^{(H)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 2.
2. A DNA<sup>(L)</sup> fragment encoding a polypeptide having nitrile hydratase activity, said polypeptide comprising the  $\alpha^{(L)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 3 and the  $\beta^{(L)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 4.
3. The DNA<sup>(H)</sup> fragment of claim 1 which contains the nucleotide sequences of the  $\alpha^{(H)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 5 and the  $\beta^{(H)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 6.
4. The DNA<sup>(L)</sup> fragment of claim 2 which contains the nucleotide sequences of the  $\alpha^{(L)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 7 and the  $\beta^{(L)}$ -subunit as defined in the Sequence Listing by SEQ ID: No. 8.
5. A recombinant DNA comprising a DNA<sup>(H)</sup> or DNA<sup>(L)</sup> of any one of claims 1-4 in a vector.
6. A transformant transformed with the recombinant DNA of claim 5.
7. A method of producing nitrile hydratase which comprises culturing the transformant as claimed in claim 6 and recovering nitrile hydratase from the culture.
8. A method of producing amides which comprises hydrating nitriles using nitrile hydratase obtained from the culture of the transformant of claim 6.
9. A method of producing amides which comprises culturing the transformant as claimed in claim 6, and hydrating nitriles to amides using the resultant culture, isolated bacterial cells, treated matter thereof, or a fixed material thereof.

FIG. 1

